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Population differentiation and recombination in wheat scab populations of *Gibberella zeae* from the United States

KURT A. ZELLER,*‡ ROBERT L. BOWDEN† and JOHN F. LESLIE*
**Department of Plant Pathology and †USDA-ARS Plant Science and Entomology Research Unit, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, Kansas 66506-5502, USA*

Abstract

In limited previous studies of the Ascomycete fungus *Gibberella zeae* in North America, the populations examined were genetically and phenotypically diverse and could be viewed as subsamples of a larger population. Our objective in this study was to test the hypothesis that a homogeneous, randomly mating population of *G. zeae* is contiguous throughout the central and eastern United States across a span of several years. We analysed presence/absence alleles based on amplified fragment length polymorphisms (AFLPs) at 30 loci, 24 of which are defined genetically on a linkage map of *G. zeae*, from > 500 isolates in eight field populations from seven states collected during the 1998, 1999 and 2000 cropping seasons. All these strains had AFLP profiles similar to those of standard isolates of *G. zeae* phylogenetic lineage 7. All the populations are genetically similar, have high genotypic diversity and little or no detectable genetic disequilibrium, and show evidence of extensive interpopulation genetic exchange. Allele frequencies in some of the populations examined are not statistically different from one another, but others are. Thus, the populations examined are not mere subsamples from a single, large, randomly mating population. Geographic distance and genetic distance between populations are correlated significantly. The observed differences are relatively small, however, indicating that while genetic isolation by distance may occur, genetic exchange has occurred at a relatively high frequency among US populations of *G. zeae*. We think that these differences reflect the time required for the alleles to diffuse across the distances that separate them, because relatively little linkage disequilibrium is detected either in the population as a whole or in any of the individual subpopulations.

Keywords: AFLPs, barley, gene flow, genetic diversity, fungi, *Fusarium graminearum*, random mating

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Introduction

In natural ecosystems, genetic, spatial and temporal variability is relatively high in host-plant populations (reviewed in Finckh & Wolfe 1997). In contrast, agricultural ecosystems are characterized by plant communities and populations that are relatively uniform, both genetically and spatially, and that are disturbed regularly by human activities (reviewed in Burdon *et al*. 1989). A plant pathogen may encounter widespread and abundant suitable habitat (hosts) in an agricultural ecosystem that would be encountered rarely in a natural ecosystem. However, individual habitat patches (fields) in agricultural ecosystems may change radically within and between growing seasons due to management practices such as crop rotation. Such management practices result in booms in the pathogen population, when a suitable host is present, and busts in pathogen populations when a suitable host is absent. In such ecosystems, local populations should regularly experience bottlenecks that can reduce local genetic and genotypic variability in these populations. Without a significant amount of genetic exchange these populations

Correspondence: John F. Leslie. Fax: + 1 785 532 2414; E-mail: jfl@ksu.edu.
‡Present address: USDA-CPHST, Plant Germplasm Quarantive and Biotechnology Laboratory, BARC-East, Building 580, Beltsville, MD 20705, USA.

can diverge to appear genetically distinct, and may maintain relatively high levels of linkage disequilibrium (Slatkin 1985).

Gibberella zeae (Schwein.) Petch (*Fusariom graminearum Schwabe*) is one of several fungal plant pathogens that cause scab, or Fusarium head blight (FHB), of wheat and of barley (Gale 2003), and has caused > $3 billion dollars of losses in Canada and the United States (McMullen *et al*. 1997; Windels 2000). Recent changes in management practices have greatly increased the airborne inoculum available to initiate FHB epidemics (Bai & Shaner 1994; Francl *et al*. 1999). While local populations from individual fields could still fluctuate in size or even, periodically, become extinct, the pool of migrants available to colonize fields has presumably been greatly increased. Studies of *G. zeae* with vegetative compatibility group (VCG) analysis (Bowden & Leslie 1992), random amplified polymorphic DNA (RAPD) markers (Dusabenyagasani *et al*. 1999; Walker *et al*. 2001), and amplified fragment length polymorphism (AFLP) markers (Zeller *et al*. 2003) have all shown that local populations of *G. zeae* within North America are genetically diverse. Two *G. zeae* populations from the northern and central Great Plains analysed with the same set of AFLP markers used in the present study were not only genetically diverse, but apparently were subsamples of a larger, randomly mating population with unspecified boundaries in both time and space (Zeller *et al*. 2003).

Our objective in this study was to test the hypothesis of Zeller *et al*. (2003) that a homogeneous, randomly mating population of *G. zeae* is contiguous throughout the central and eastern United States across a span of several years. Our working hypothesis was that sufficient genetic exchange occurred between local populations for there to be no significant regional or temporal partitioning of allelic variation among them. This study provides a broad-based baseline population description against which future collections from the United States or other regions can be compared.

Materials and methods

Strain isolation and identification

G. zeae populations from individual wheat fields were sampled in 1998, 1999 and 2000, as permitted by the regionally and annually patchy nature of FHB disease outbreaks (Table 1). In three populations, KS1, KS2 and MN, we collected symptomatic wheat heads separated by at least 3 m along sets of parallel transects at least 3 m apart. For the five other populations there was no information on the spatial sampling pattern. The OH sample was received as wheat seed heads, and the other four samples were received as bulk seed from combine-harvested fields. In populations derived from samples composed of individual heads, no more than one *Fusarium* isolate was taken from a head, and in the other samples no more than one isolate was taken from a wheat kernel.

For head-based populations, we isolated *Fusarium* by removing a single symptomatic kernel and plating it directly onto a semiselective peptone PCNB medium (Nash &

Table 1 Collection data and estimated disease incidence for eight *Gibberella zeae* populations from the United States

Population	Location	Year	Host	FHB incidence	*Fusarium* isolates*		Collector
					Total no.	% *G. zeae*	
KS1	Ashland, Kansas	May, 1999	Various winter wheat cultivars and breeding lines	< 1%	90	93	Kurt A. Zeller
KS2	Holton, Kansas	May, 1999	Hard red winter wheat variety 2137	8%	71	100	Kurt A. Zeller
MN	Perley, Minnesota	July, 1999	Hard red spring wheat, cultivar unknown	< 1%	72	94	Robert L. Bowden
VA	Blacksburg, Virginia	June, 1998	Soft red winter wheat, cultivar unknown	Not reported	72	83	Erik. Stromberg
IL	Elkville, Illinois	June, 1999	Soft red winter wheat, cultivar unknown	Not reported	71	79	Robert Frank
NY	Pittsford, New York	July, 2000	Soft white winter wheat, cultivar Caledonia	17%†	94	80	Gary C. Bergstrom
MT	Scobey, Montana	July, 2000	Durum wheat, cultivar Kyle	9%†	72	51	Robert Johnston
OH	Croton, Ohio	June, 1999	Soft red winter wheat, cultivar OH 515	< 1%	75	97	Patrick E. Lipps

**G. zeae* isolates were identified initially by morphology (Nelson *et al*. 1983), and by self-fertility on carrot agar. Identification as *G. zeae* was confirmed by AFLP fingerprint profile comparisons.
†Estimated by the provider of these samples as the incidence of *Fusarium* species recovered from these seed samples.

Snyder 1962). For seed lot-based populations, we isolated *Fusarium* directly from discolored or tombstone kernels by plating ≥ 120 kernels/population sample on the peptone-PCNB medium. Seeds on peptone-PCNB medium were incubated for 4–7 days at 25 °C with a 12-h:12-h light:dark cycle, which allowed the *Fusarium* isolate(s) to grow out onto the medium. Colonies were subcultured onto a complete medium (Correll *et al*. 1987) slant. Once these subcultures conidiated, single macroconidia were separated by micromanipulation, and the resulting cultures stored as spore/hyphal fragment suspensions in 15% glycerol at –70 °C.

We initially identified putative *G. zeae* isolates by morphology, and by testing for self-fertility on carrot agar (Klittich & Leslie 1988; Bowden & Leslie 1999) and/or carnation leaf agar (Fisher *et al*. 1982). Isolates lacking *G. zeae* morphological characters were not included in the subsequent AFLP fingerprint analyses. We confirmed putative isolates as *G. zeae* by comparison of AFLP fingerprint profiles with those of isolates KSU 05047 (= FGSC 8631, phylogenetic lineage 6) and KSU 03639 (= FGSC 8630) and KSU 03634 (both representatives of phylogenetic lineage 7). These three isolates were included as amplification and AFLP profile standards on all gels, and had constant AFLP fingerprint profiles that were independent of digestion/ligation reactions, AFLP preamplification reactions and the DNA preparation/extraction process. We also compared AFLP fingerprint profiles of the field isolates with those of representative strains from *G. zeae* phylogenetic lineages 1–5 (O'Donnell *et al*. 2000). Not all isolates could be run on a single gel, so isolates from up to four source populations commonly were run in blocks of no more than 30/population on single gels to minimize scoring ambiguities among geographically separated populations.

DNA manipulation methods and AFLP analyses

We cultured strains routinely, extracted DNA and stored and assessed DNA concentration and purity as outlined in Kerènyi *et al*. (1999). AFLPs (Vos *et al*. 1995) were generated as described by Zeller *et al*. (2000) using the AFLP primes combinations utilized by Zeller et al. (2003). We used all buffers and DNA modifying enzymes following the manufacturer's instructions or standard protocols (Sambrook *et al*. 1989). *Eco*RI primers were end-labelled for the final specific PCR amplifications with γ^{33}P-ATP and AFLP fragments were resolved in 6% denaturing polyacrylamide (Long Ranger, FMC Scientific, Rockland, ME) gels in 1× TBE buffer (pH 8.3). We exposed dried gels to autoradiography film (Classic Blue Sensitive, Molecular Technologies, St Louis, MO) for 2–7 days at room temperature to visualize banding patterns. We estimated band sizes on polyacrylamide gels against γ^{33}P-labelled BRL Low-mass ladder (BRL Life Technologies, Rockville, MD).

We scored manually the presence or absence of polymorphic AFLP bands representing DNA fragments between 100 and 800 base pairs (bp) in length, and recorded the allelic data (presence or absence) in a binary format. AFLP marker alleles are dominant (band present)/recessive (band absent), but can be scored unambiguously in *G. zeae* because this fungus is haploid. We assumed that bands with the same mobility in different individuals were homologous and represented the same allele. Unresolved bands or missing data were coded as ambiguous. Bands used in the population genetic analyses were polymorphic among the set of isolates KSU 03639 (from wheat in Kansas), KSU 03634 (from wheat in Kansas), KSU 05047 (from barley in Japan) and KSU 11609 (lineage 6, from barley in South Korea). AFLP loci polymorphic between KSU 03639 and KSU 05047 can be placed on the genetic linkage map of Jurgenson *et al*. (2002). The AFLP loci used in these population genetic analyses also were polymorphic in population KS1 and were a subset of those used to analyse populations from Kansas and North Dakota (Zeller *et al*. 2003), with the frequency of the rarer allele (i.e. the presence or absence of a band) ≥ 0.05. We used the mapped genetic markers to evaluate genetic disequilibrium within *G. zeae* populations between both linked and unlinked loci.

Haplotype diversity assessments and population genetic analyses

We identified indistinguishable *G. zeae* AFLP haplotypes (putative clones) within populations by analysing the binary data with unweighted pair grouping by mathematical averaging (UPGMA) clustering with the program PAUP* 4.10b (Swofford 1999). All but one representative isolate of each haplotype from a population was censored from the population genetic analyses. We estimated genotypic diversity ($\hat{G}$) for each population as described by Milgroom (1996) and normalized the index for each population by dividing each estimated $\hat{G}$ by the number of genotypes identified from that population.

We estimated (i) allele frequencies at polymorphic loci and genetic diversity within and between populations as described by Nei (1973), (ii) G_{ST} (fixation index) and (iii) *Nm* (effective migration rate) as described by Nei (1987), and (iv) genetic identity among populations as described by Nei (1978) with the shareware program POPGENE version 1.32 (available at http://www.ualberta.ca/~fyeh, Yeh *et al*. 1997). All data were analysed by this program as haploid, dominant markers. We also used POPGENE to detect linkage disequilibrium across 30 loci (Brown *et al*. 1980) and to conduct χ^2 tests for significance and calculations for two-locus gametic disequilibria between all pairs of loci in all eight clone-censored populations (Weir 1979).

The statistical significance of Φ_{ST} (an estimate of the fixation index) values and 95% confidence intervals

of the observed differentiation among individual and regional populations were evaluated with 1000 bootstrap resamplings of the data in the software GDA (Genetic Data Analysis; Lewis & Zaykin 2001). Analysis of molecular variance (AMOVA) analyses (Excoffier *et al*. 1992) also were conducted with GDA to examine the partitioning of genetic variation among regional and temporal populations and subpopulations. We ran Pearson correlation analyses between estimated Φ_{ST}, *Nm*, genetic identity, geographical separation (km) and log-transformed geographical distances between sampling sites with the CORR procedure of SAS (version 6.12) (SAS Institute, Cary, NC, USA).

Results

G. zeae *recovery and population diversity*

We examined 617 *Fusarium* isolates from FHB-infested wheat. The frequency of *G. zeae* isolates from among these *Fusarium* isolates in individual samples ranged from a high of 100% in KS2 to a low of 51% in MT (Table 1). *G. zeae* was recovered at a higher rate (*t*-test, $P < 0.05$) from infested whole wheat heads (96%) than from seed-lot samples (73%). Other morphological species of *Fusarium* recovered in addition to *G. zeae* (*F. graminearum*) included *F. culmorum*, *F. equiseti*, *F. poae*, *F. proliferatum* and *F. sporotrichioides*.

We ran AFLPs on 523 *G. zeae* strains (Table 2). Individual populations included between 36 (MT) and 84 (KS1) *G. zeae* isolates, so as a function of the binomial probability equation we can be 95% certain that we detected all of the alleles present at > 8% frequency in the MT population and > 3.5% frequency in the KS1 population.

Genotypic diversity and recombination

Normalized genotypic diversity ($\hat{G}$) was high (≥ 95% of the count) in all eight populations, with the highest number of clonal genotypes recovered from the IL population. The highest genotypic diversity (100%) was from the MT population, in which all 36 isolates had unique AFLP haplotypes (Table 2).

There is no evidence for significant linkage disequilibrium between the AFLP loci within any of these populations. The number of polymorphic AFLP loci in single populations ranged from 26 to 30 (Table 2). In all eight populations, the estimated s_k^2 value (variance between observed and expected heterozygosity for pairs of loci) ranged from 0.52 to 2.05 (data not shown). In all cases these values were less than the estimated 95% cut-off values for significance (range 6.75–8.14, data not shown), and we could not reject the hypothesis of random mating (Brown *et al*. 1980). The percentage of locus pairs that were significantly different from equilibrium expectations in the eight populations (χ^2 test, $P < 0.05$) ranged from a minimum of 3.4% (15/435 pairs) in the OH population to a maximum of 8.7% (38/435 pairs) in the IL population.

Population differentiation

The overall estimated G_{ST} among the eight populations averaged across 30 loci that we calculated with POPGENE was $G_{ST} = 0.0411$, with a range from 0.0069 for locus *EAAMAT0393K* to 0.1665 for locus *EAAMAT0106J*. This range suggests that there has been significant genetic exchange among populations of *G. zeae* (*Nm* averaged > 12 across the 30 loci) across the central and eastern United States wheat-growing region. Despite these strong indications of gene flow among populations, allele frequencies for 16/30 AFLP loci differed among populations (χ^2 goodness of fit, $P < 0.05$).

Pairwise estimates of the fixation index (Φ_{ST}, Table 3) among the eight populations were uniformly low and ranged from 0.004 (KS1/KS2) to 0.067 (IL/MT), and estimates of effective migration number (*Nm*) derived from

Table 2 Estimates of genotypic diversity ($\hat{G}$) in eight clone-censored populations of *Gibberella zeae* based on 30 polymorphic loci

Population	Isolates	Unique haplotypes	No. of polymorphic loci	$\hat{G}$*	$\hat{G}/n$†
KS1	84	81	30	78.4	0.968
KS2	71	69	29	67.2	0.974
IL	56	51	30	49.0	0.961
MN	68	64	30	60.8	0.950
OH	73	72	28	71.1	0.988
VA	60	58	28	56.3	0.971
NY	75	72	28	69.4	0.964
MT	36	36	26	36.0	1.000

*$\hat{G}$ was calculated as described in Milgroom (1996) from comparisons of AFLP allelic data at 30 AFLP loci. $\hat{G} = 1/\Sigma p_i^2$ where p_i = the observed frequency of the ith multilocus genotype in a population.

†$\hat{G}/n$ was calculated by dividing $\hat{G}$ by the number of AFLP haplotypes observed in each population.

Table 3 Pairwise estimates of fixation index (Φ_{ST}) and effective migration number (*Nm*) based on 30 AFLP loci from eight populations of *Gibberella zeae*. Φ_{ST} values calculated by GDA (Lewis & Zaykin 2001) are in the lower left portion of the table, and *Nm* values (McDermott & McDonald 1993) are in the upper right portion of the table

	KS1	KS2	IL	MN	OH	VA	NY	MT
KS1	—	132	74	16	32	18	31	7
KS2	0.004	—	68	22	52	13	29	11
IL	0.007	0.007	—	9	44	19	23	7
MN	0.030*	0.022	0.051*	—	14	19	19	17
OH	0.016*	0.010*	0.011	0.035*	—	22	23	9
VA	0.027*	0.031*	0.025*	0.025*	0.022*	—	14	8
NY	0.016	0.017*	0.021*	0.026*	0.022*	0.033*	—	10
MT	0.065*	0.045*	0.067*	0.028*	0.051*	0.059*	0.049*	—

*The value is statistically > 0 ($P < 0.05$) based on 1000 bootstrap replications.

Table 4 UPGMA similarity calculated as Nei's unbiased genetic identity (Nei 1978) based on 30 AFLP loci (lower left), and geographical separation* (km) for eight *Gibberella zeae* populations (upper right)

	KS1	KS2	IL	MN	OH	VA	NY	MT
KS1	—	74	658	891	1194	1428	1649	1271
KS2	0.995	—	594	868	1121	1360	1574	1287
IL	0.993	0.991	—	1202	619	778	1140	1772
MN	0.983	0.986	0.971	—	1369	1737	1573	665
OH	0.990	0.992	0.989	0.980	—	386	532	2025
VA	0.985	0.981	0.981	0.983	0.986	—	692	2384
NY	0.978	0.977	0.978	0.971	0.977	0.972	—	2233
MT	0.933	0.941	0.922	0.954	0.942	0.933	0.923	—

*Geographic distances separating sites were estimated with Distance Calculations software (http://www.zipmath.com), converted from miles to km, and rounded to the nearest km.

Table 5 Estimates of fixation index (Φ_{ST}), and of statistical confidence for Φ_{ST} values among regional subdivisions of *Gibberella zeae*

Population subdivision	Φ_{ST} among populations	Φ_{ST} among subpopulations within regional populations
Among all eight populations	0.025*	NA
All populations except MT	0.022*	NA
1998/1999 vs. 2000	0.034*	0.057*
North† vs. south‡	0.005	0.025*
Spring[3] vs. winter[4] wheat	0.061*	0.079*

*Indicates a value that is statistically > 0 ($P < 0.05$) based on 1000 bootstrap replications as implemented in the program GDA (Lewis & Zaykin 2001).
†Includes populations MN, MT and NY.
‡Includes populations IL, KS1, KS2, OH and VA.
[3]Includes populations MN and MT.
[4]Includes populations IL, KS1, KS2, NY, OH, and VA.

those Φ_{ST} values were correspondingly high (Table 3). Pairwise estimates of genetic identity (Table 4) between populations also were uniformly high, ranging from 0.959 (IL/MT) to 0.995 (KS1/KS2).

The estimate for Φ_{ST} across the 30 AFLP-defined loci for all eight populations was 0.025 (Table 5) and was statistically different from zero. If the data for the most genetically divergent and most geographically isolated population (MT) are removed, then Φ_{ST} decreases to 0.022 (Table 5), but remains statistically different from zero. These data indicate that > 97% of the allelic variance is shared among populations and < 3% is due to allelic variance between

separate populations. When populations are compared to one another in all possible pairwise combinations, 6/28 pairwise comparisons are not significantly different from zero (Table 3). These six comparisons include the pairwise combinations of KS1, KS2 and IL, and single pairwise comparisons between populations KS1 and NY, KS2 and MN, and IL and OH. The mean distance between these population pairs was 744 km (SD = 516), compared to the overall mean of 1181 km (SD = 567) for all 28 population pairs. Even the two populations with the greatest pairwise difference (IL and MT) shared > 93% of allelic variation (Table 3). Estimates of Φ_{ST} among populations grouped north vs. south ($\Phi_{ST} = 0.005$) were slightly positive, but were not significantly greater than zero (Table 5). However, we did detect some evidence for low ($\Phi_{ST} = 0.034$), but statistically significant partitioning of allelic variation among populations grouped by year of collection (1998/1999 vs. 2000), and among populations from spring vs. winter wheat ($\Phi_{ST} = 0.061$; Table 5). Thus there are no diagnostic differences at the AFLP loci examined that can be used to track isolates to a region of origin within the central and eastern United States.

Correlations between genetic and geographical distance

There were significant correlations between estimates of population similarity and geographical separation between populations. There was a highly significant negative correlation ($r = -0.59$, $P < 0.001$) between estimates of population identity (Nei 1978) and distances between populations (Fig. 1). There also were highly significant correlations between estimated fixation indices (Φ_{ST}) and geographical separation ($r = 0.616$, $P < 0.001$, data not shown), and between estimated effective migration number (Nm) and geographical separation ($r = -0.572$, $P < 0.002$, data not shown).

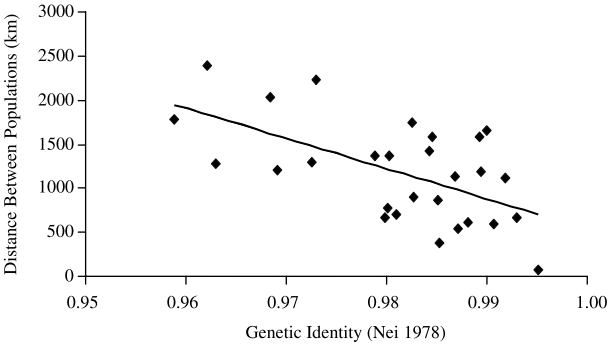

Fig. 1 Correlation between estimated genetic identity (Nei 1978) and physical distance between *Gibberella zeae* populations ($r = -0.591$; $P < 0.001$).

Discussion

G. zeae *phylogenetic lineages recovered*

The AFLP profiles of all the *G. zeae* isolates examined are consistent with those produced by strains representing phylogenetic lineage 7 (O'Donnell *et al*. 2000), which is expected if a single interbreeding population of *G. zeae* causes FHB in North America (Dusabenyagasani *et al*. 1999; Zeller *et al*. 2003). A single interbreeding population also appears to dominate in *G. zeae* populations causing FHB in China, but significant linkage disequilibrium was present in this interbreeding population and the strains in the Chinese interbreeding population belong to phylogenetic lineage 6 (Gale *et al*. 2002). Whether other populations of *G. zeae* from other regions of the world share this relatively simple phylogenetic structure remains to be tested rigorously.

Recombination in G. zeae *populations*

Data consistent with recombination in field populations of fungal plant pathogens has been reviewed by Milgroom (1996) and McDermott & McDonald (1993). In many cases the reported recombination was extensive and a randomly mating population structure could not be excluded. Recombination has occurred in field populations of *G. zeae*, although we cannot determine whether outcrossing and recombination occurs regularly or episodically. All eight of our tested populations have high estimated genotypic diversity (> 95% of that possible, Table 2) and there are relatively few locus pairs in gametic disequilibrium in these populations. Unlike Zeller *et al*. (2003), we found no evidence for linkage disequilibrium in the region that includes the *TRI* gene cluster. Thus, hypotheses of random mating and/or recombination cannot be excluded for field populations of *G. zeae* in the United States.

Recombination, and its effects on population dynamics, have important consequences for understanding the epidemiology and evolutionary potential of *G. zeae* in agriculture. FHB in the United States is thought to be initiated primarily by ascospores (Bai & Shaner 1994; Markell & Francl 2003), but could also be spread by contaminated grain. The relatively high VCG, molecular marker and pathogenic diversity observed both in the United States and elsewhere, e.g. Schilling *et al*. (1997), Dusabenyagasani *et al*. (1999), Moon *et al*. (1999), Walker *et al*. (2001) and Zeller *et al*. (2003), are all consistent with a hypothesis of at least episodic recombination and long-distance dispersal. This high variation is somewhat surprising, as many of the ascospores probably are formed in a homothallic manner in which no recombination or genetic exchange occurs. Because similar pictures of population structure and composition result when different sampling methods are used, the critical factor in analysing *G. zeae* populations probably

is sample size rather than sampling protocol, as long as reasonable precautions are taken to reduce multiple samples of vegetatively propagated strains. Schilling *et al*. (1997), Gilbert & Tekauz (2000) and Zeller *et al*. (2003) all reported that multiple infections of individual wheat heads by *G. zeae* were common. The genotypic diversity observed could be explained if ascospores are the dominant cause of primary FHB infection; if multiple, genetically different strains can infect the same wheat or maize plant; and if these strains outcross even sporadically.

Population differentiation and evidence for isolation by distance

There are a few other large-scale studies of population dynamics of fungal plant pathogens that have utilized allelic data from molecular markers. For example, El-Touil *et al*. (1999) observed small, but statistically significant, differences between subpopulations from eastern Canada of *Cronartium ribicola* based on allelic data from RAPD markers, which they attributed to drift following local extinction and recolonization. They detected neither regional differentiation between subpopulations nor correlation between geographical and genetic distance and concluded that long-distance dispersal was an epidemiologically important factor in blister rust dynamics. Zhan *et al*. (2003) determined that > 90% of the global allelic diversity of *Mycosphaerella graminicola*, based on RFLP alleles, could be found in single wheat fields, and that < 5% and < 3% of the variation was unique to fields within regions and to regions, respectively. They postulated that global populations were linked by gene flow, either current or historic, but found a negative correlation between *Nm* and geographical distance that led them to suggest an Island Model of dispersal (Wright 1951).

The *G. zeae* populations we examined (Table 5) have low, but statistically significant, levels of allelic difference ($\Phi_{ST} < 0.03$); however, even the two populations with the greatest pairwise difference (IL and MT) shared > 93% of allelic variation (Table 3). Some of these small statistical differences may be attributable to sampling error and the high statistical power generated through analysis of 30 relatively balanced polymorphic loci and from comparing populations of ≥ 36 individuals. These differences could also be attributed to genetic drift among subpopulations following local extinction and recolonization events, but the relative lack of genetic disequilibrium in any of these samples argues against this hypothesis.

The difference between populations grouped according to their spring (MN and MT populations) or winter wheat sources (Table 5) is positive ($\Phi_{ST} = 0.061$) and statistically different from zero, as is Φ_{ST} among subpopulations within each agronomic type (Table 5). No other significant differences were detected between any other regional groups of populations examined. These data may be interpreted as a temporal lag in gene-flow, or as a result of selection for cropping system specialization that has resulted in a slightly greater divergence among these groupings. Temporally, the six populations from 1998/1999 and the two from 2000 (NY and MT) (Table 5) are statistically different. However, even these differences are relatively small, with only an estimated 3.4% of the allelic variation partitioned between sampling years, and 6.1% of the allelic variation partitioned between spring and winter wheat samples (Table 5). These results suggest that gene flow occurs, or has occurred, between *G. zeae* populations, but that diffusion of new alleles within the greater population is not instantaneous.

The hypothesis that free gene-flow among regional populations of *G. zeae* has short-term limits also is consistent with a correlation between genetic distance and physical distance (Fig. 1). Within the United States, regional differentiation among populations appears minimal; however, temporal differences indicate that movement of novel alleles among populations may take time. These cropping system-related, and temporal differences have not been targets of previous studies and are worthy of more detailed investigation. In addition, studies of populations from durum wheat, barley, maize and rice from geographically contiguous locations are needed to determine if there are differences in the *G. zeae* populations associated with any of these crops relative to those from spring and winter wheat.

In conclusion, these results are consistent with those of previous work (Dusabenyagasani *et al*. 1999; Zeller *et al*. 2003), who found $G_{ST} < 0.038$ in *G. zeae* populations from different sampling years, and $0.01 < G_{ST} < 0.051$ for populations from different locations. Thus, the existing data suggest that North American populations of *G. zeae* have participated in considerable genetic exchange, either currently or historically. If pathogenicity and aggressiveness genes are distributed in a manner similar to the molecularly detected genetic variation, then resistance breeding materials planted at any single location should be exposed to a representative range of the pathogenic variation present in the continental *G. zeae* population, and differences between locations should probably be attributed to factors other than differences in genetic composition of the pathogen population.

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The authors are interested in the genetics and distribution of *Fusarium* species in both native and agricultural ecosystems. With *Gibberella zeae* they have constructed genetic maps, made field collections from countries on all continents and begun testing the hypothesis that the species is composed of genetically distinct lineages. This study provides baseline genetic information for the United States against which similar collections from other locations can be critically evaluated.